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Variants of *Triticum mosaic virus* Isolated From Wheat in Colorado Show Divergent Biological Behavior

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Abstract

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Triticum mosaic virus (TriMV) is a recently discovered virus infecting wheat. We compared Colorado isolates C10-492 and C11-775 with the 06-123 isolate. Comparisons were made using enzyme-linked immunosorbent assay (ELISA), infectivity assay, host range, dry weight (DW), inoculation of 'Mace' wheat with temperature-sensitive resistance to *Wheat streak mosaic virus*, and the deduced amino acid sequence of the coat proteins (CP) and P1 proteins. Both C10-492 and C11-775 infected 'Gallatin' barley and, when compared with 06-123, had significantly reduced ELISA values and virus titers in wheat. Both Colorado isolates caused symptomless infections in Mace, whereas 06-123 caused mosaic symptoms. The amino acid sequences of the CP differed by two and one amino acids for C10-492 and C11-775, respectively, compared with 06-123. The sequence of C10-492 differed from C11-775 by one amino acid. The P1 amino acid sequence of C10-492 and C11-775 differed from 06-123 by three and one amino acids, respectively. The C10-492 and C11-775 isolates reduced DW significantly in 'Karl 92' but significantly less than 06-123. In '2317' wheat, the Colorado isolates did not consistently cause significant reduction in DW, while 06-123 did. The data collectively indicate that C10-492 and C11-775 are isolates of TriMV showing biological behavior diverse from that of 06-123.

Triticum mosaic virus (TriMV) was first identified infecting wheat (*Triticum aestivum* L.) in Kansas in 2006 (17). TriMV has since been identified from infected wheat in the Great Plains in Colorado, Nebraska, Oklahoma, South Dakota, Texas, and Wyoming (1). Field surveys were conducted in Colorado, Kansas, Nebraska, and South Dakota in spring and fall 2010 and 2011 to determine TriMV incidence and the frequency of its co-infection with *Wheat streak mosaic virus* (WSMV) or High Plains virus (HPV) in winter wheat (2). That study showed that TriMV was detected in all four states and that WSMV was the most prevalent virus, followed by TriMV and HPV. Furthermore, 91% of TriMV-positive samples were co-infected with WSMV, whereas WSMV and HPV were mainly detected as single infections. The virus is mechanically transmissible, and has a coat protein (CP) of approximately 35 kDa. Antiserum raised to the CP of TriMV reacted only with this virus in enzyme-linked immunosorbent assay (ELISA) and Western blot assay (17). Symptomatic plants were shown to be associated with flexuous rods when analyzed by electron microscopy (17).

The sequence of the CP of the 06-123 isolate of TriMV determined by time-of-flight mass spectroscopy and reverse-transcription polymerase chain reaction (RT-PCR) showed that TriMV was distantly related to *Sugarcane streak mosaic virus* (17). The complete nucleotide sequences of the Kansas 06-123 (5) and a Nebraska isolate (21) and partial sequences of other Kansas, Oklahoma, and Texas isolates of TriMV have been determined (6).

Wheat curl mites (WCMs; *Aceria tosichella* Keifer) have been demonstrated to transmit TriMV both singly and in combination with WSMV (16). Thus, in addition to TriMV, the WCM also transmits WSMV (19) and the HPV (11).

Previously, only naturally infected wheat and barley have been confirmed as hosts of TriMV (17). The reaction of row crop species to infection by TriMV and WSMV and the identification of differential hosts for these viruses have been conducted (13,14,20).

Infection of wheat by TriMV was demonstrated to cause significant yield losses in replicated field trials at Hays, Kansas in 'Danby', 'RonL', and 'Jagalene' wheat but not the wheat line KS96HW10-3 (15). In a greenhouse study, yield determinants such as tillers per plant and shoot and root weight were reduced in 'Millennium' but not 'Mace' wheat when singly infected with TriMV or doubly infected with TriMV and WSMV (3).

Wheat samples C10-492 (collected in Colorado in 2010) and C11-775 (collected in Colorado in 2011) with virus-like symptoms reacted weakly (low ELISA values for TriMV) with antibodies against TriMV and against WSMV but not with those against HPV. We report characteristics of these isolates when compared with the original 06-123 isolate of TriMV isolated in Kansas in 2006 (17) and show that field-collected Colorado viruses are isolates of TriMV showing divergent biological characteristics.

Materials and Methods

Infectivity assays. 'Tomahawk' wheat, 'Arcia' triticale, 'Gallatin' barley, and 'N28Ht' maize were used as the assay plants and inoculated mechanically (finger rub) at the single-leaf stage for wheat, barley, and triticale and at the two-leaf stage for maize, with 1:10 (wt/vol) extracts prepared from appropriate infected wheat sources (as described above). Following inoculation, the plants were held in a growth chamber at 22°C (as described above) for 14 or 21 days post inoculation (DPI) and numbers of symptomatic plants were recorded.

Virus source and maintenance. The 06-123 isolate of TriMV was isolated from KS06HW79 wheat in 2006 at the Kansas Agri-

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The C10-492 and C11-775 CP sequences have been assigned the GenBank accession numbers JX843399 and JX843400, respectively, and the P1 sequences KC262645 and KC262644, respectively.

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cultural Research Center–Hays (KSU-ARCH) at Hays, KS (17). The C10-492 isolate of TriMV was obtained from a leaf sample of 'Storm' wheat growing in Baca County, CO on 17 May 2010. C11-775 was isolated from a leaf sample of an unidentified wheat cultivar in an unidentified county in Colorado in 2011. In the original ELISA analyses, both leaf samples C10-492 and C11-775 reacted with TriMV and WSMV antibodies. TriMV-reactive isolate was separated from the C10-492 field sample by passaging through Gallatin barley and then Arcia triticale, and then propagated in Tomahawk wheat. The C11-775 virus was passaged once through Arcia and then to Tomahawk wheat. Gallatin barley and Arcia triticale have been shown to be capable of infection by the 06-123 isolate of TriMV but not the Sidney 81 isolate of WSMV (14). Both the C10-492 and C11-775 isolates were propagated in Tomahawk wheat by mechanical inoculation as described previously (17). Following inoculation, the plants were held in a greenhouse under natural lighting. Infected plants were harvested and frozen at –80°C until needed for sources of inoculum for other studies. Once propagated in Tomahawk wheat after passaging through Gallatin barley and Arcia triticale, the C10-492 and C11-775 samples did not react with antibodies of WSMV and reacted weakly to antibodies of TriMV.

Sources of antisera. The TriMV antiserum was used as a whole serum at a 1:2,000 (vol/vol) dilution prepared from a stock solution of protein at 1 mg/ml and was raised as described previously (17). The WSMV (1:1,000 [vol/vol]) (10) and HPV antiserum (1:1,000 [vol/vol]) (HPV-WO) (8) dilutions were made from stocks adjusted to protein at 1 mg/ml. Positive controls consisted of the 06-123 isolate of TriMV (16), the Sidney 81 isolate of WSMV (17), and the 06-Maize A isolate of HPV (8).

Direct antigen coating ELISA. Direct antigen coating (DAC)-ELISA was performed essentially as described previously (17). Briefly, plant tissue was ground in a 1:30 (wt/vol) dilution of coating buffer (4) in a 1.5-ml microcentrifuge tube using a wooden applicator stick (Fisher Scientific). Extracts (200 µl) were placed in separate wells of ELISA plates (Immulon 1; Fisher Scientific) for 1 h at 37°C. The wells were rinsed and then incubated for 1 h at 37°C with the appropriate antivirus antibody (as described above) in dilution buffer (4). Following incubation, the wells were rinsed and each well was blocked by adding 0.4 ml of blocking buffer (5% nonfat dry milk, 0.01% antifoam A, and 0.02% sodium azide in phosphate-buffered saline, pH 7.4) and held for 1 h at 37°C. Then, 200 µl of anti-rabbit antibody/alkaline phosphatase conjugate (Southern Biotechnology Associates) in dilution buffer (1:3,000 [vol/vol]) was added to each well. The plates were held at 37°C for 1 h. The plates were rinsed, and 200 µl of p-nitrophenyl phosphate substrate at 0.714 mg/ml in substrate buffer (4) was added to each well. The plates were then held on a bench top at 20 to 22°C for 30 min. Absorbance was measured at 405 nm using an iMark plate reader (Bio-Rad Laboratories). Absorbance values were arbitrarily considered positive if they were two times greater than the healthy value (GHV). For the purposes of brevity, rather than repeatedly stating that extracts from plants reacted with an antibody at a GHV of 2 or greater, we state that such a plant or plants were positive in ELISA and plants below that GHV were negative in ELISA.

Host, titer, and infectivity assay analyses of TriMV isolates. *Propagation of isolates of TriMV.* Seed of Tomahawk wheat were planted in separate rows in three soil-filled (Harney clay loam soil, fine montmorillonitic, mesic type Argiustoll) flats (21 by 35 cm), each with seven rows and 8 to 10 seeds planted in each row. The planted flats were held in a growth chamber (Percival Model PGC-15WC) set at 22°C with a 12-h photoperiod of fluorescent light (250 $\mu Es^{-1}\ m^{-2}$) until the plants were at the single-leaf stage. The plants were mechanically (finger rub) inoculated with a 1:10 (wt/vol) dilution of extract prepared from single Tomahawk wheat plants testing positive in ELISA against antibodies of TriMV only**.** The inoculated plants were held in a growth chamber set at 22°C with a 10-h photoperiod of fluorescent light (250 $\mu Es^{-1}\ m^{-2}$). At 14 DPI, the plants were separately harvested for each isolate and several 1-g portions prepared for each isolate and frozen at –80°C for future use.

Planting and inoculation procedures. Seed of the maize line N28Ht, the barley Gallatin, and the wheat Mace and Tomahawk were planted in three separate rows for each seed source in a soil-filled (Harney clay loam soil, fine montmorillonitic, mesic type Argiustoll) flats (35 by 50 cm), with 11 rows planted with 8 to 10 seeds each. Plants of these species were separately inoculated with 1:10 (wt/vol) dilutions of extract for each virus isolate. At the same time, an additional flat of the same size with 11 rows each was divided in half, so that 22 rows were planted with seed of Tomahawk to provide plants for inoculation with serial dilutions of extract prepared from Tomahawk wheat separately infected with the C11-775, C10-492, or 06-123 isolates. These two planted flats were held in a room under 16 h of fluorescent light (100 $\mu Es^{-1}\ m^{-2}$) until the plants were at the single-leaf stage. The maize, barley, Mace, and Tomahawk wheat plants were mechanically (finger rub) inoculated with a 1:10 (wt/vol) dilution of extract prepared from Tomahawk wheat plants infected with the appropriate virus as described above. From the 1:10 (wt/vol) extracts of each virus isolate, separate 1:25 (vol/vol) dilutions of extract were prepared and, from each of these 1:25 (vol/vol) dilutions, 1:50 (vol/vol), 1:100 (vol/vol), 1:200 (vol/vol), 1:400 (vol/vol), and 1:800 (vol/vol) serial dilutions were prepared for each isolate. Each of these diluted extracts (1:25 to 1:800 [vol/vol]) was used to inoculate separate rows of Tomahawk wheat plants. These inoculated plants were held in a chamber set at 22°C with a 10-h photoperiod of fluorescent light (250 $\mu Es^{-1}\ m^{-2}$). Numbers of symptomatic plants were recorded at 14 DPI. At 14 DPI, 10 individual third leaves of Tomahawk wheat assay plants that had been inoculated with the 1:10 (wt/vol) extracts were individually harvested for each isolate. Each leaf was divided in half and the tip portion of each leaf bulked, weighed, and analyzed separately by ELISA at a 1:30 (wt/vol) dilution (as described above). The remaining leaf pieces were bulked within an isolate and used as the inoculum source to prepare a 1:10 (wt/vol) extract, from which 1:400 (vol/vol) and 1:800 (vol/vol) dilutions were prepared. These two dilutions were used to mechanically (finger rub) inoculate Tomahawk wheat assay plants at the single-leaf stage. The Tomahawk wheat assay plants were held in a room at 22°C (±4°C) with a 16-h photoperiod of fluorescent light (100 $\mu Es^{-1}\ m^{-2}$) and numbers of symptomatic plants were recorded at 21 DPI. The experiment was conducted three times. The analysis of variance of the data for the three experiments was conducted using SAS (version 8; SAS Institute) and significant treatment effects were determined using the least significant difference test at $P = 0.05$.

Dry weight analyses. The C11-775, C10-492, and 06-123 isolates of TriMV were used for analyses of dry weight of virus-infected wheat. Each isolate was propagated as described above. Several 1-g (four to five plants required to weigh 1 g) samples were prepared for each isolate and the samples frozen at –80°C until needed.

Three metal flats (35 by 50-cm) were filled with 12.7 kg of soil (Harney clay loam, fine montmorillonitic, mesic type Argiustoll). Each flat had 14 rows, with the centers of the rows approximately 35 mm apart, and the rows were divided in half, resulting in 28 rows, each 150 mm long. The outer two rows on the side of each flat were planted with Tomahawk wheat as filler so that all other rows of wheat had wheat growing on each side of them. The remaining 24 rows were planted with sieved seed (KSU-ARCH sources) of '2137' and 'Karl 92' wheat, using seed that were collected on a 2.7-by-19-mm sieve. Seed sources were randomly assigned to the 24 rows. This scheme of planting allowed for four rows planted for each cultivar to which treatments (mock inoculated, C11-775, C10-492, or the 06-123) were randomly assigned. Each flat served as one of three replications. Following planting, the flats were held in a chamber (Percival Model PGC-15WC) at 22°C with a 10-h photoperiod of fluorescent light (250 $\mu Es^{-1}\ m^{-2}$) until the plants were at the single-leaf stage. Each row of wheat was then thinned to 10 plants of uniform size in both experiments.

Following thinning, the plants were mechanically inoculated with a 1:10 (wt/vol) extract of the appropriate virus prepared from the frozen virus sources. Following inoculation, the flats of plants were randomly placed in a chamber (Percival Model PGC-15WC) at 22°C with a 10-h photoperiod of fluorescent light as described above. After 28 days, the plants in each row were harvested separately by cutting each plant off at ground level with scissors, and the plants were bulked, weighed, and placed in labeled paper bags. The bags were then placed in a forced-air dryer at 76°C and dried for 7 days to a constant weight. Following drying, the plants in each bag were weighed. The experiment was conducted two times, each with three replications. The analysis of variance of the data (symptom ratings and dry weight) for each experiment was conducted using SAS (version 8; SAS Institute) and significant treatment effects were determined using the least significant difference test at $P = 0.05$. In our dry weight studies, we chose Karl 92 and 2137, both susceptible to WSMV, because Karl 92 showed severe symptoms in demonstration plots in the field and 2137 showed a mild mosaic when mechanically inoculated with the 06-123 isolates (D. L. Seifers, *unpublished*).

Nucleic acid sequencing of the TriMV P1 and CPs. Tomahawk wheat used for sequencing analyses was obtained from wheat separately infected with the 06-123, C10-492, and C11-775 viruses obtained from the samples prepared as described for propagation of isolates of TriMV above. Total RNA was extracted from wheat leaves as described previously (9). The total RNA was used to generate first-strand cDNA using random hexamer primers (Promega Corp.) and *Avian myeloblastosis virus* (AMV) reverse transcriptase (Roche). The P1 and CP cistrons were amplified using cDNA as a template, with primer combinations of Tr-72 (nucleotides corresponding to 301 to 318) and Tr-76 (nucleotides complementary to 1,888 to 1,859), and Tr-99 (nucleotides corresponding to 9,195 to 9,223, with an *Nco*I restriction site) and Tr-100 (nucleotides complementary to 10,078 to 10,049, with an *Xho*I restriction site), respectively. The PCR program consisted of a single cycle at 94°C for 2 min; 35 cycles at 94°C for 20 s, 54°C for 20 s, and 72°C for 90 s; followed by a single cycle at 72°C for 5 min. The gel-isolated RT-PCR products were directly sequenced at the University of Florida ICBR Core DNA Sequencing Facility using an Applied Biosystems 3730 model sequencer.

Results

Separation of TriMV from WSMV in the original wheat tissue. The original C10-492 wheat leaf extract from Storm reacted with antibodies of both TriMV and WSMV in ELISA. The values for TriMV and WSMV were 0.286 and 0.592, respectively, while the values for healthy wheat for the TriMV and WSMV antiserum were 0.027 and 0.042, respectively. The extract prepared from the original C10-492 wheat sample was used to inoculate Tomahawk wheat and Gallatin barley (Table 1). Six of eight Tomahawk wheat plants became systemically infected in this first transfer. Four of the plants were positive for both TriMV and WSMV and had a low mean ELISA value for TriMV of 0.067 (range 0.024 to 0.086) and a mean of 0.284 (range 0.058 to 0.510) for WSMV. The two other symptomatic plants were positive for only WSMV, with a mean ELISA value of 0.308 (range of 0.289 to 0.327). The single symptomatic Gallatin barley plant was positive for TriMV only and had an ELISA value of 0.145. This barley plant provided the inoculum to inoculate Tomahawk wheat and Gallatin barley in the second transfer.

In the second transfer, the extract prepared from the barley plant infected eight Tomahawk wheat plants (Table 1). Seven of the wheat plants were positive for TriMV only and had a mean ELISA value of 0.118. One wheat plant was positive for both TriMV and WSMV. For this plant, the ELISA value for WSMV was 0.034, which was only 2.3 times larger than the healthy control, and it had a value of 0.126 for TriMV antiserum. The single plant testing weakly positive for WSMV when used to inoculate Tomahawk wheat was not infective for WSMV, only TriMV. None of the 10 barley plants inoculated in the second transfer developed symptoms. The 06-123 control in wheat had an ELISA value of 0.606.

Leaf tissue from the seven Tomahawk wheat plants from the second transfer of C10-492 was bulked and used as the inoculum source to inoculate Tomahawk wheat, Arcia triticale, and N28Ht maize in a third transfer experiment (Table 1). None of the four and five maize plants that were inoculated with the extracts from plants infected with C10-492 and 06-123 isolates of TriMV, respectively, were infected (*data not shown*). The four maize plants inoculated with the Sidney 81 isolate of WSMV all became systemically infected (*data not shown*). All 10 wheat plants developed mosaic symptoms and two of six Arcia triticale plants developed symptoms. The nine symptomatic wheat plants with values above the positive threshold had a mean ELISA value of 0.116 for the TriMV antiserum, with a range of 0.098 to 0.126. These ELISA values were between 2.1 and 2.7 times the 0.047 value of the healthy control. The other symptomatic wheat plant had a value of 0.089 that was 1.9 times the healthy control and was not included in the nine positive plants in Table 1 and not used in the next experiment. The two triticale plants had a mean ELISA value of 0.113, and ELISA values of 0.372 and 0.434 were recorded for the 06-123 TriMV and Sidney 81 WSMV controls, respectively.

Table 1. Numbers of plants of 'Tomahawk' wheat, 'Gallatin' barley, and 'Arcia' triticale whose extracts reacted with antibodies of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or both viruses following mechanical inoculation with extracts from wheat sample C10-492[y]

Transfer	TriMV	WSMV	TriMV & WSMV	TriMV	WSMV	TriMV & WSMV
1	0/8	Tomahawk 2/8 (0.308)	4/8 (0.067) (0.284)[z]	**1/8** (0.145)	Gallatin 0/8	0/8
2	**7/8** (0.118)	Tomahawk 0/8	1/8 (0.126) (0.034)[z]	0/10	Gallatin 0/10	0/10
3	9/10 (0.116)	Tomahawk 0/10	0/10	**2/6** (0.113)	Arcia 0/6	0/6

[y] Numbers in parenthesis are mean enzyme-linked immunosorbent assay (ELISA) values. Numbers in bold are the plants used as the source of inoculum for the following experiment. For example, the single barley plant from the first transfer was used to prepare the inoculum used to mechanically inoculate Tomahawk wheat and Gallatin barley seedlings in the second transfer of the C10-492 isolate. The two triticale plants infected in the third transfer were used as the source to propagate the isolate in Tomahawk wheat for use in other studies. Only extracts of symptomatic plants reacted with antibodies of TriMV or WSMV.

[z] The first number in parentheses is the mean TriMV ELISA value and the second is the mean WSMV ELISA value.

Table 2. Numbers of plants of 'Tomahawk' wheat and 'Arcia' triticale whose extracts reacted with antibodies of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or both viruses following mechanical inoculation with extracts from wheat sample C11-775[z]

Transfer	TriMV	WSMV	TriMV & WSMV	TriMV	WSMV	TriMV & WSMV
1	0/8	Tomahawk 0/9	0/8	**1/9** (0.104)	Arcia 0/9	0/9
2	**4/7** (0.090)	Tomahawk 0/7	0/7	0/10	Arcia 0/10	0/10

[z] Numbers in bold are the plants used as inoculum sources. For example, the single triticale plant from the first transfer was used to prepare the inoculum used to mechanically inoculate Tomahawk wheat and triticale in the second transfer of the C11-775 isolate. The four wheat plants infected in the second transfer were used as the source to propagate the isolate in Tomahawk wheat for use in other studies. Only extracts of symptomatic plants reacted with antibodies of TriMV or WSMV. Numbers in parentheses are mean enzyme-linked immunosorbent assay values.

The original wheat leaf sample C11-775 reacted to antibodies against TriMV (ELISA value of 0.058) and WSMV (ELISA value of 0.155) compared with the healthy control value of 0.017. In the first infectivity assay experiment, the extract prepared from the original tissue infected one of nine Arcia triticale plants with TriMV but none of the eight Tomahawk wheat plants were infected (Table 2). Extract from this triticale plant had a low TriMV ELISA value of 0.104 compared with the 06-123 control value of 0.544 and a healthy value of 0.010. Extract from this triticale plant was used in the second infectivity assay experiment to inoculate Arcia triticale and Tomahawk wheat. In that assay, none of the 10 triticale plants were infected but 4 of 7 Tomahawk wheat plants were infected. The symptomatic Tomahawk wheat plants had low ELISA values of 0.095 (GHV 9.5), 0.068 (GHV 6.8), 0.096 (GHV 9.6), and 0.102 (GHV 10.2), respectively. The mean ELISA value for these four plants was 0.090, similar to the original wheat tissue. The mean ELISA value (from six symptomatic wheat plants) for the 06-123 isolate control was 0.456 and the healthy wheat control was 0.005. These four wheat plants were bulked and used as the inoculum source to propagate the C11-775 isolate in Tomahawk wheat for further studies. None of the Tomahawk plants inoculated with the extract prepared from the four wheat plants reacted with antibodies against WSMV (*data not shown*).

Host and dilution assays. Inoculation of N28Ht maize, Gallatin barley, and Mace wheat with three isolates of TriMV resulted in infection of plants depending on species (Table 3). None of the isolates infected maize. All isolates infected Gallatin barley but the numbers of infected barley plants were significantly lower for the C11-775 and C10-492 isolates when compared with the 06-123 isolate. One symptomatic Mace wheat plant was observed following inoculation with the C11-775 isolate. Two symptomatic plants were observed when Mace wheat was inoculated with the C10-492 isolate. However, symptoms of the Mace plants inoculated with C11-775 and C10-492 consisted of a few chlorotic dashes approximately 1 to 2 mm in length, and Mace plants inoculated with extracts of 06-123 had a prominent mosaic. The 06-123

Table 3. Percentages (means from three experiments) of symptomatic 'N28Ht' maize, 'Gallatin' barley, and 'Mace' and 'Tomahawk' wheat plants following mechanical inoculation with different isolates of *Triticum* mosaic virus (TriMV) when held at 22°C for 14 days

Species	Virus[v]	Dilution[w]	*N*[x]	NSP[y]	Infection (%)[z]
N28Ht maize	C11-775	1:10	26	0	0.0 I
N28Ht maize	C10-492	1:10	29	0	0.0 I
N28Ht maize	06-123	1:10	28	0	0.0 I
Gallatin barley	C11-775	1:10	44	18	40.9 DEF
Gallatin barley	C10-492	1:10	43	18	41.8 DE
Gallatin barley	06-123	1:10	36	32	88.8 BC
Mace wheat	C11-775	1:10	46	1	2.2 HI
Mace wheat	C10-492	1:10	44	2	4.5 GH
Mace wheat	06-123	1:10	45	39	86.6 AC
Tomahawk wheat	C11-775	1:10	32	32	100 A
Tomahawk wheat	C11-775	1:25	33	33	100 A
Tomahawk wheat	C11-775	1:50	30	29	96.6 A
Tomahawk wheat	C11-775	1:100	28	26	92.8 A
Tomahawk wheat	C11-775	1:200	30	19	63.3 CD
Tomahawk wheat	C11-775	1:400	31	6	19.3 EFG
Tomahawk wheat	C11-775	1:800	27	0	0.0 I
Tomahawk wheat	C10-492	1:10	30	30	100 A
Tomahawk wheat	C10-492	1:25	32	32	100 A
Tomahawk wheat	C10-492	1:50	32	32	100 A
Tomahawk wheat	C10-492	1:100	27	26	96.2 A
Tomahawk wheat	C10-492	1:200	29	22	75.8 BC
Tomahawk wheat	C10-492	1:400	29	9	31 EF
Tomahawk wheat	C10-492	1:800	29	5	17.2 GF
Tomahawk wheat	06-123	1:10	30	30	100 A
Tomahawk wheat	06-123	1:25	28	28	100 A
Tomahawk wheat	06-123	1:50	31	31	100 A
Tomahawk wheat	06-123	1:100	30	30	100 A
Tomahawk wheat	06-123	1:200	25	25	100 A
Tomahawk wheat	06-123	1:400	30	28	93.3 A
Tomahawk wheat	06-123	1:800	31	22	71 C

[v] The C before the isolate numbers indicates Colorado. The number 10 indicates 2010, 11 = 2011, and 06 = 2006; the number following the dash (-) is the plant sample number.
[w] The 1:10 dilution is wt/vol and the other dilutions were prepared from the 1:10 and are vol/vol. The same 1:10 (wt/vol) dilution (for a given virus isolate) was used to inoculate the maize, barley, and Mace and Tomahawk wheat (1:10 [wt/vol]) plants.
[x] Total numbers of plants mechanically inoculated in three experiments.
[y] Numbers of systemically infected plants.
[z] Treatments not having a letter in common are significantly different using the least significant difference test ($P = 0.05$). Coefficient of variation is 16.2%.

Table 4. Numbers of symptomatic plants (SPs) of 'Mace' wheat and numbers of plants with extracts that reacted with antibodies of *Triticum mosaic virus* (TriMV) in enzyme-linked immunosorbent assay (ELISA) analyses of the plants

Treatment	*N*[x]	SP	ELISA positive[y]	ELISA mean[z]	ELISA minimum[z]	ELISA maximum[z]
C11-775	48	1	3	0.007	0.001	0.030
C10-492	44	2	5	0.011	0.003	0.055
06-123	45	43	43	0.171	0.021	0.769
Healthy wheat	1	0	0	0.008	0.008	0.009

[x] Number of plants inoculated in three experiments.
[y] Number of plants whose extracts reacted with antibodies of TriMV in ELISA so that the value was at least twice that of the healthy wheat control value.
[z] Mean value of all plants considered positive in ELISA for a given virus treatment. The minimum or maximum ELISA values for plants considered positive in ELISA for a given virus treatment.

isolate infected 39 of 45 Mace plants and most of them had a prominent mosaic. The 1:10 (wt/vol), 1:50 (vol/vol), and 1:100 (vol/vol) dilutions for each virus isolate infected 92.8 to 100% of Tomahawk wheat plants, so that no significant differences among the isolates were observed. However, the 1:800 (vol/vol) was significantly different among the three isolates, with the 06-123 isolate having 71.1% infected Tomahawk wheat plants and the C11-775 isolate 0% (Table 3).

When each Mace wheat plant was analyzed by ELISA, some symptomatic plants were not considered positive in ELISA (Table 4). The symptomatic Mace plant inoculated with the C11-775 isolate was positive in ELISA, with a value of 0.022 (2.4 times greater than the healthy control value). In all, 2 of 44 plants inoculated with the C10-492 isolate developed symptoms (few dashes); 1 of these 2 plants had a value of 0.019, which was less than the positive threshold, and the other had a value of 0.055, 6.1 times greater than the healthy control. For the 06-123 isolate, 43 of 45 plants inoculated developed symptoms; 41 had prominent mosaic, 2 had dashes only, and all were positive in ELISA. The ELISA values for plants considered positive for the C11-775 and C10-492 isolates were very low in contrast to (except for the two plants with only small chlorotic dashes 1 to 2 mm long for symptoms) the plants inoculated with the 06-123 isolate. Interestingly, for the C10-492 and C11-775 isolates, some Mace plants rated as symptomless had ELISA values that were considered positive.

The observation about symptomless plants with ELISA values that were considered positive prompted an evaluation of Mace plants testing both positive and negative in ELISA that may or may not have been rated as symptomatic (Table 5). For the C11-775 isolate, the symptomatic plant was infective to Tomahawk wheat and all plants rated as symptomless (except one) were infective, although some were negative in ELISA. For Mace plants inoculated with the C10-492 isolates, the two symptomatic plants were infective, although one was considered negative in ELISA. All symptomless plants inoculated with the C10-492 isolate, whether positive or negative in ELISA, were infective. For the Mace plants inoculated with the 06-123 isolate, all were rated as symptomatic and had a wide range of ELISA values and all were highly infective, as were the external controls for each isolate.

ELISA analyses of 1:30 (wt/vol) extracts prepared from half of the third leaf of Tomahawk wheat inoculated with the C11-775, C10-492, and 06-123 isolates of TriMV showed significant differences in values (Table 6). All ELISA values for the isolates were significantly different from the healthy control, except for the C11-775 isolate, although the ELISA value for this isolate was eight times more than the healthy control value. The value for the C10-

Table 5. Numbers of symptomatic 'Tomahawk' wheat assay plants when held at 22°C for 21 days post inoculation (DPI), following mechanical inoculation with extracts prepared from 'Mace' wheat that was inoculated with different isolates of *Triticum mosaic virus* (TriMV)

				Symptomatic Tomahawk wheat	
Symptoms[w]	**Isolate**	**ELISA[x]**	**GHV[y]**	**At 21 DPI[z]**	**Infection (%)**
NS	C11-775	0.016	1.8	0/11	0.0
NS	C11-775	0.030	3.3	3/11	27.3
NS	C11-775	0.022	2.4	3/11	27.3
Faint mosaic	C11-775	0.022	2.4	8/9	88.9
NS	C11-775	0.017	1.9	1/9	11.1
Dashes	C10-492	0.017	1.9	10/10	100.0
NS	C10-492	0.015	1.7	5/9	55.6
NS	C10-492	0.018	2.0	1/10	10.0
Dashes	C10-492	0.055	6.1	10/10	100.0
NS	C10-492	0.018	2.0	9/10	90.0
NS	C10-492	0.020	2.2	9/9	100.0
NS	C10-492	0.028	3.1	8/11	72.7
Mosaic	06-123	0.574	63.8	10/10	100.0
Mosaic	06-123	0.068	7.6	8/8	100.0
Dashes	06-123	0.021	2.3	10/10	100.0
Mosaic	06-123	0.203	22.6	10/10	100.0
Mosaic	06-123	0.207	23.0	6/6	100.0
Dashes	06-123	0.019	2.1	7/10	70.0
Mosaic	C11-775	Control	NA	10/10	100.0
Mosaic	C10-492	Control	NA	11/11	100.0
Mosaic	06-123	Control	NA	11/11	100.0

[w] Symptoms are those of the individual Mace wheat plant that provided the inoculum for the Tomahawk wheat infectivity assay. NS = no symptoms.

[x] Enzyme-linked immunosorbent assay (ELISA) values are for a given Mace wheat plant that provided the inoculum for the Tomahawk wheat infectivity assay. Control = additional samples of Tomahawk wheat infected with each of the isolates of TriMV but not from the same experiment as the Mace wheat samples.

[y] Number of times greater than the healthy control value for a given ELISA value. An ELISA value of 2.0 was arbitrarily considered as positive in the ELISA of the Mace plants.

[z] Numerator represents the number of symptomatic Tomahawk wheat assay plants and the denominator the total number of plants inoculated.

Table 6. Mean enzyme-linked immunosorbent assay (ELISA) values and mean percentages of infected plants resulting from different dilutions of extracts prepared from the third leaf of 'Tomahawk' wheat plants infected with different isolates of *Triticum mosaic virus* (TriMV) when held at 22°C for 14 days[z]

		Plant extract dilution		
Treatment	**ELISA**	**1:30 (wt/vol)**	**1:400 (vol/vol)**	**1:800 (vol/vol)**
C11-775	0.073 CB	27 C	0 D	0 D
C10-492	0.126 B	41 BC	0 D	0 D
06-123	0.504 A	100 A	55 B	27 C
Healthy wheat	0.009 C	…	…	…

[z] Means were calculated using ELISA values (1:30 [wt/vol]) from three experiments. C10-492 and Cll-775 isolates of TriMV were collected from wheat in Colorado in 2010 and 2011 and the 06-123 isolate of TriMV from wheat in Kansas in 2006. Treatment means (ELISA means are compared separately from means for infectivity assays) in a column for the ELISA analyses and within and between columns for infectivity analyses not followed by the same letter are significantly different using the least significant difference test ($P = 0.05$). The coefficient of variation for ELISA and infectivity analyses was 22.8 and 23.9%, respectively.

492 isolate was different from the 06-123 value but not C11-775. The 06-123 value was significantly more than ELISA values of the other two Colorado isolates.

We compared the infectivity of different dilutions of extract prepared from the other half of the third leaves used for the ELISA analyses in an infectivity assay to Tomahawk wheat. Numbers of plants infected with the 1:30 (wt/vol) and 1:400 and 1:800 (vol/vol) dilutions were significantly different depending upon isolate (Table 6). The 1:30 (wt/vol) extracts prepared from tissue inoculated with the three isolates were infective and the numbers of infected plants for the 06-123 isolates were significantly larger. The 1:400 and 1:800 extracts for 06-123 were infective, whereas those for C11-775 and C10-492 were not. The numbers of infected plants for the 1:400 and 1:800 dilutions for 06-123 were different.

Dry weight analyses. Each isolate of TriMV caused a significant reduction in dry weight accumulation in the Karl 92 when compared with the mock-inoculated control but this was not observed for 2137 (Table 7). In both experiments, the dry matter accumulation for Karl 92 was significantly less in plants inoculated with 06-123 when compared with the other isolates, which were not different from each other. In experiment 1 for 2137, the values for the 06-123 isolate were significantly different from the mock-inoculated control but not those of the other two isolates, whose values were not different from the mock-inoculated control. In the second experiment with 2137, the 06-123 isolate had a value that was significantly smaller than that of the mock-inoculated control but was not different from that of C10-492, whose value was not different from that for C11-775. The value for C11-775 was also not different from that of the mock-inoculated control.

Nucleic acid sequencing of the TriMV P1 and CPs. The comparison of the deduced amino acid sequence of the CP of the C10-492 and C11-775 isolates showed differences at the N termini compared with the 06-123 isolate (Fig. 1). For the C10-492 sequence, two differences occurred at positions 17 and 26. At posi-

Table 7. Effect of infection of different wheat varieties on dry weight accumulation following mechanical inoculation with different isolates of *Triticum mosaic virus* (TriMV) when held at 22°C for 28 days[z]

		Experiment 1		Experiment 2	
Wheat	**Treatment**	**Dry weight (g)**	**Reduction (%)**	**Dry weight (g)**	**Reduction (%)**
Karl 92	C11-775	1.212 BC	36	1.147 DE	41
Karl 92	C10-492	1.118 BC	41	1.076 E	44
Karl 92	06-123	0.791 D	59	0.683 F	65
Karl 92	Mock inoculated	1.893 A	…	1.915 A	…
2137	C11-775	1.154 BC	10	1.394 BC	8
2137	C10-492	1.169 BC	8	1.293 CD	15
2137	06-123	1.063 C	17	1.146 DE	25
2137	Mock inoculated	1.269 B	…	1.516 B	…

[z] Dry weight values are means calculated from three replications in each experiment. Percent reduction for the inoculated treatments was calculated based on the mock-inoculated values for each wheat cultivar. Treatment means not followed by the same letter differ according to the least significant difference test at $P = 0.05$. Means are compared within a column only. Coefficient of variation = 8.7 and 8.8% for experiments 1 and 2, respectively.

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          1                                                          60

06-123    SGADQSGVVK DQTGDKAEGS GTKTEDPPNQ TTDPVNNPSN GGNKDAPQNL NATVVTKSYT
C10-492   SGADQSGVVK DQTGDKTEGS GTKTEGPPNQ TTDPVNNPSN GGNKDAPQNL NATVVTKSYT
C11-775   SGADQSGVVK DQTGDKTEGS GTKTEDPPNQ TTDPVNNPSN GGNKDAPQNL NATVVTKSYT

         61                                                         120
06-123    YIPPIMKSLV TIDTAKKMAD YTPPDALIST QACTLEQFGR WANAAANGLG LSMQAFQTDV
C10-492   YIPPIMKSLV TIDTAKKMAD YTPPDALIST QACTLEQFGR WANAAANGLG LSMQAFQTDV
C11-775   YIPPIMKSLV TIDTAKKMAD YTPPDALIST QACTLEQFGR WANAAANGLG LSMQAFQTDV

        121                                                         180
06-123    VPYWIYWCIV NSASDEHKKL SSWTKVNMTI DDATGQINLN EGEAQTIYEM SPMFDEAKPT
C10-492   VPYWIYWCIV NSASDEHKKL SSWTKVNMTI DDATGQINLN EGEAQTIYEM SPMFDEAKPT
C11-775   VPYWIYWCIV NSASDEHKKL SSWTKVNMTI DDATGQINLN EGEAQTIYEM SPMFDEAKPT

        181                                                         240
06-123    LRAVMRHFGA LAYRWVKFSI AKRKPIIPHN AIKAGLMDVT YFPCCIDFVT VDQLSPQEQN
C10-492   LRAVMRHFGA LAYRWVKFSI AKRKPIIPHN AIKAGLMDVT YFPCCIDFVT VDQLSPQEQN
C11-775   LRAVMRHFGA LAYRWVKFSI AKRKPIIPHN AIKAGLMDVT YFPCCIDFVT VDQLSPQEQN

        241                                                    294
06-123    VRNQVINARV SDTPRALFKH AQRAGAGEED TNLRRDDDAN YGRTRVGGAM FGTR
C10-492   VRNQVINARV SDTPRALFKH AQRAGAGEED TNLRRDDDAN YGRTRVGGAM FGTR
C11-775   VRNQVINARV SDTPRALFKH AQRAGAGEED TNLRRDDDAN YGRTRVGGAM FGTR
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Fig. 1. Amino acid sequences of the coat proteins of *Triticum mosaic virus* (TriMV) as determined by computer translation of the nucleotide sequences. The sequence of the C10-492 and C11-775 Colorado isolates (JX843399 and JX843400) of TriMV are aligned with that of the Kansas 06-123 isolate (EF 173696). Differences in amino acids occur at the positions 17 and 26 and are underlined.

tion 17, the amino acid alanine was replaced with threonine and, at position 26, the aspartic acid residue was replaced with a glycine in C10-492. For the C11-775 isolate, only one difference existed at position 17, where the amino acid alanine present in 06-123 was replaced with threonine. Only one difference at position 26 occurred between C10-492 and C11-775, where the amino acids glycine and aspartic acid occurred for C10-492 and C11-775, respectively.

The amino acid sequence of the P1 protein differed among the isolates. The differences occurred at positions 188,189, 237, and 305 (Fig. 2). At position 188, the amino acids of the Colorado isolates differed from each other, with C10-492 having a glycine residue while C11-775 had an arginine, as did 06-123. At position 189, the amino acids of the Colorado isolates differed from each other by C11-775 having the amino acid cysteine and C10-492 tyrosine, as did 06-123. At position 237, both Colorado isolates had the amino acid lysine and 06-123 had arginine. At position 305, both C11-775 and 06-123 had the amino acid glutamic acid and C10-492 had glutamine. Thus, the sequence of the C10-492 and C11-775 isolates differed from that of 06-123 by three and two amino acids, respectively.

Discussion

TMV has been reported in many of the states in the Great Plains (1,2). To date, little variation among isolates with respect to the CP has been reported (6,21) and no variation of any kind for biological behavior among isolates of TriMV has been reported. In this study, we found that the virus isolates C10-492 and C11-775 from Colorado had much lower ELISA values compared with those of the 06-123 isolate first discovered in Kansas in 2006 (17). The values were low enough that we considered the possibility that they might not have been isolates of TriMV. We examined the relationship of ELISA analyses to infectivity, host effects, and ability to infect Mace wheat that has temperature-sensitive resistance to WSMV (7), and the amino acid sequence of the viral CP and P1 proteins of the C10-492 and C11-775 isolates with that of the 06-123 isolate.

The original wheat samples C10-492 and C11-775 collected in Colorado were positive in ELISA for both TriMV and WSMV. These original samples had low ELISA values using antibodies of TriMV compared with the 06-123 control. However, no special significance was given to the low ELISA values at that time because the condition of both samples was poor; therefore, the virus

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      10                                                          70
06-123  MSSKKMMWVP KSAHKAPVVS REPVIRKKEW VARQIPKYIP VSNPSDCRDE ISQTLLHFDS
C10-492 MSSKKMMWVP KSAHKAPVVS REPVIRKKEW VARQIPKYIP VSNPSDCRDE ISQTLLHFDS
C11-775 MSSKKMMWVP KSAHKAPVVS REPVIRKKEW VARQIPKYIP VSNPSDCRDE ISQTLLHFDS


       71                                                         130
06-123  EEAVYDFVWR FPMGSIFWDT NGRIKPVVNC LLRATRMNLD YDVAADVYVC RDCLSCASSY
C10-492 EEAVYDFVWR FPMGSIFWDT NGRIKPVVNC LLRATRMNLD YDVAADVYVC RDCLSCASSY
C11-775 EEAVYDFVWR FPMGSIFWDT NGRIKPVVNC LLRATRMNLD YDVAADVYVC RDCLSCASSY


      131                                                         190
06-123  MYFSNYHYDC RELRENHEAV VSCKYEQHIV STFDVFPRYC TQEIEQNVVN WMTETLERYD
C10-492 MYFSNYHYDC RELRENHEAV VSCKYEQHIV STFDVFPRYC TQEIEQNVVN WMTETLEGYD
C11-775 MYFSNYHYDC RELRENHEAV VSCKYEQHIV STFDVFPRYC TQEIEQNVVN WMTETLERCD


      191                                                         250
06-123  NEPLRIEKQL QFYNHKTEQM ESRVQEVQVT TAEYAVSDTY VPQQLSRKGS VSAKLTQRRA
C10-492 NEPLRIEKQL QFYNHKTEQM ESRVQEVQVT TAEYAVSDTY VPQQLSLKGS VSAKLTQRRA
C11-775 NEPLRIEKQL QFYNHKTEQM ESRVQEVQVT TAEYAVSDTY VPQQLSLKGS VSAKLTQRRA


      251                                                         310
06-123  NKIIMRTHEV ENLIRETIDL CDERQIPITF VDVKHKRCLP RIPLRHMQAK PDISEIVEQG
C10-492 NKIIMRTHEV ENLIRETIDL CDERQIPITF VDVKHKRCLP RIPLRHMQAK PDISQIVEQG
C11-775 NKIIMRTHEV ENLIRETIDL CDERQIPITF VDVKHKRCLP RIPLRHMQAK PDISEIVEQG


      311                                                         370
06-123  DMYNEVGQFI EQYQNLAEPF RVIRDYEVTR GWSGVILHRD DLALDPQTQA RCLNNLFVVM
C10-492 DMYNEVGQFI EQYQNLAEPF RVIRDYEVTR GWSGVILHRD DLALDPQTQA RCLNNLFVVM
C11-775 DMYNEVGQFI EQYQNLAEPF RVIRDYEVTR GWSGVILHRD DLALDPQTQA RCLNNLFVVM


      371          381
06-123  GRCEHGHLQN A
C10-492 GRCEHGHLQN A
C11-775 GRCEHGHLQN A
```

Fig. 2. Amino acid sequences of the P1 proteins of isolates of *Triticum mosaic virus* (TriMV). The sequence of the C10-492 (KC262645) and C11-775 (KC 262644) Colorado isolates of TriMV is aligned with that of the Kansas 06-123 (FJ263671). Differences in amino acids occur at the positions 188, 187, 237, and 305 and are underlined.

might have degraded during the transport or conditions in the field where these samples were collected were different, so that no direct comparison of ELISA values for these samples with 06-123 was valid. However, in the following transfers (Tables 1 and 2) of these isolates to separate them from WSMV, we noted that ELISA values for the Colorado isolates using antibodies of TriMV remained lower in wheat when compared with the 06-123 isolates.

We further investigated this phenomenon, comparing the C10-492, C11-775, and 06-123 isolates in an experiment where different hosts were inoculated with a 1:10 (wt/vol) dilution of each isolate, and that same dilution was used to inoculate Tomahawk wheat, along with 1:400 and 1:800 dilutions that were prepared from the 1:10 (wt/vol) dilution. We showed that none of the isolates infected N28Ht maize, a species previously shown not to be a host of TriMV (14). We noted that, in the second transfer of the C10-492 isolate, none of the Gallatin barley plants were infected (Table 1). However, Gallatin barley, a species shown to be a host of TriMV (14), was infected with each isolate in the host range study (Table 3). Interestingly, Mace wheat, with temperature-sensitive resistance to WSMV (7), showed significant differences in the numbers of symptomatic plants present following inoculation, with both the C10-492 and C11-775 isolates having fewer plants with symptoms, which consisted only of a few dashes. The high numbers of symptomatic plants of Mace (mostly prominent mosaic) infected with the 06-123 isolate has been observed previously (D. L. Seifers, *unpublished*).

This prompted us to analyze all the Mace plants grown at 22°C by ELISA using antibodies of TriMV. We found that symptomless plants inoculated with the C10-492 and C11-775 isolates reacted with the TriMV antibodies, although at low levels compared with the Mace plants inoculated with the 06-123 isolate (Table 4). We further compared certain of these plants in infectivity assay inoculations to Tomahawk wheat (Table 5). Here, we observed that extracts from plants inoculated with the C10-492 and C11-775 isolates that were symptomless but positive in ELISA were infective, as were symptomless plants that were negative in ELISA. This was not totally unexpected because low ELISA values associated with Tomahawk wheat inoculated with the Colorado isolates had been continually observed from the first analysis of the original C10-492 and C11-775 wheat samples. However, these results with Mace showed that, compared with the 06-123 isolate, the C10-492 and C11-775 isolates are much different in their ability to cause symptom formation in this cultivar. In another study using a Nebraska isolate (isolated from wheat 22 May 2008 in Red Willow County) (21) of TriMV, when Mace wheat was inoculated, mild symptoms were observed at 19°C, with pronounced symptoms at 20 to 26°C in a greenhouse (20). In that same study, it was shown that TriMV replicated in Mace poorly at 14 DPI but, by 28 DPI at 20 to 26°C, it replicated efficiently.

We also separately compared the three isolates by ELISA using antibodies of TriMV and infectivity of extracts from leaf three of the Tomahawk wheat plants that were inoculated with the 1:10 (wt/vol) extracts presented in the comparative analyses in Table 3. We observed that the ELISA values for the 1:30 (wt/vol) extracts were significantly lower for the C10-492 and C11-775 isolates when compared with 06-123. The ELISA analysis was performed at a 1:30 (wt/vol) dilution and should not have been in a linear dilution range for virus concentration, and one would not have expected such findings. It was shown with wheat infected with WSMV that the linear response range, at least for infectivity analyses, was between 1:100 and 1:600 (vol/vol) dilutions (12). Thus, the ELISA results strongly suggested that the Colorado isolates might be isolates of TriMV with low titer in wheat. We confirmed the low titer hypothesis by using the 1:400 (vol/vol) and 1:800 (vol/vol) dilutions, where none of the Tomahawk assay plants were infected with the extracts prepared from plants inoculated with the Colorado isolates. In contrast, when these dilutions were prepared from plants infected with the 06-123 isolate, they were infective.

Comparison of the complete CP amino acid sequence of the 06-123 isolate with the CP protein sequences of C10-492 and C11-775 indicated that they were isolates of TriMV (Fig. 1). We observed that the derived amino acid sequence of the RT-PCR products of the CP of the C10-492 and C11-775 isolates varied by only a few amino acids from that of the 06-123 isolate at the N termini. The C10-492 isolate differed by two amino acids from the 06-123 isolate and the C11-775 isolate by one amino acid. This is in keeping with previous findings, where the sequence of the CP of 14 isolates of TriMV collected from Kansas, Oklahoma, and Texas differed by three amino acids only (6). It was interesting to note that both of these changes in amino acids occurred in the N-terminal region of the CP. The N-terminal region is considered to be the region to contain major virus-specific epitopes of potyviruses (18). Perhaps the significantly lower titer observed in systemically infected wheat leaves for the C10-492 and C11-775 isolates compared with the 06-123 isolate is related to the two changes in amino acid observed for the C10-492 sequence, or it is possible that differences in other proteins also might contribute to low virus titer because we did not sequence the complete genome. It is also plausible that the two amino acid changes in the CP of the C10-492 isolate are not responsible for the biological differences observed in this study. However, no information exists for TriMV concerning this, and speculation about this is beyond the scope of this work. It is interesting to note that the C11-775 CP sequence differs from that of C10-492 by only one amino acid at position 26; thus, if the CP is solely involved in the low titer of the Colorado isolates, then this may be a critical observation. However, the C11-775 and 06-123 sequences have the same amino acid at this location and the 06-123 isolate has a high titer in wheat. However, attributing biological changes to specific sequence data will require development of infectious cDNA clones of TriMV and more detailed experimentation and analyses. Further biological comparisons should use these isolates that have been genetically modified at this location to prove such a point. It is also plausible that other regions of the virus genome may be involved in reduced virus titers in the Colorado isolates.

The P1 domain is reported as the least conserved and most variable in length among monopartite viruses in the family *Potyviridae* (22). The P1 protein of the 06-123 isolate of TriMV is 383 amino acids (aa) in length (5). We used 371 of the 383 aa for the sequence comparison and found that C10-492 and C11-775 differed by only 3 and 2 aa, respectively, from 06-123 (Fig. 2). Furthermore, the amino acid sequence of the P1 protein of the Red Willow isolate (FJ669487) of TriMV collected in Nebraska has been determined (21). The P1 sequence of the Red Willow isolate differs from 06-123 by one amino acid only, as does the C11-775 isolate. Interestingly, this difference occurs at position 237, where the Red Willow isolate has a leucine, as do both C10-492 and C11-775, in contrast to arginine for 06-123. These findings further support the identity of C10-492 and C11-775 as isolates of TriMV. Reduction in yield and test weight of wheat plants infected with the 06-123 isolate of TriMV has been demonstrated under field conditions (15). In that experiment, two sources of resistance to WSMV (RonL and KS96HW10-3) were compared with Danby and Jagalene, which are susceptible to WSMV. Danby, RonL, and Jagalene recorded significant yield reductions and the KS96HW10-3 line did not. In the dry weight experiments, we observed, for Karl 92, that the 06-123 isolate caused significant reduction in dry weight accumulation when compared with the mock-inoculated controls and the C10-492 and C11-775 isolates, and that the Colorado isolates also caused a significant reduction in dry weight accumulation compared with the mock-inoculated control. Thus, one could assume that the Colorado isolates would cause significant yield reductions of Karl 92 in the field. Again, with 2137, the 06-123 isolate caused significant reductions in dry matter accumulation compared with the mock-inoculated control but this was not observed in both experiments for the Colorado isolates. Thus, dry weight experiments may not be predictive of yield results for all cultivars, depending upon the isolate of TriMV, particularly those associated with low titer. Although this remains to be proven in the field, these findings indicate that, when working with TriMV, results will be influenced

by the isolate. Also, these dry weight findings indicate that Karl 92 provides more reproducible results and would be of value when comparing isolates in dry weight analyses. Reductions in shoot and root weight in the greenhouse have been demonstrated as well with a Nebraska isolate of TriMV (3).

Taken together, the data indicate that the C10-492 and C11-775 viruses are isolates of TriMV. This was verified by comparison of amino acid sequences of the CP and P1 proteins. These Colorado isolates have low ELISA values using antibodies of TriMV and show reduced titer in wheat and infect Gallatin barley, although at significantly lower levels than the 06-123 isolate. The symptomless infection of plants of Mace by the Colorado isolates, which has temperature-sensitive resistance to WSMV, was demonstrated. Thus, care needs to be taken when comparing the ability of isolates of TriMV to infect wheat with different sources of temperature-sensitive resistance. The C10-492 and C11-775 isolates reduced dry weight accumulation significantly in the Karl 92 but not 2137 wheat. This indicates that Karl 92 would be a host of choice when comparing isolates of TriMV from different regions for impact on dry weight. Further work should consider determining the prevalence of other isolates of TriMV similar to C10-492 and C11-775 and determining their effects on yield and host range when compared with isolates such as 06-123.

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